1/11/2022 Mcl

Increasing Power Generation in Benthic Microbial Fuel Cells through Supplementation with Lactate

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August 15, 2007

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Research Experience for Undergraduates

Abstract

Six laboratory-scale chambered benthic microbial fuel cells were constructed and monitored over four weeks for electrical and chemical variables. Three of the cells were supplemented weekly with lactate, and three were controls. Supplementation caused immediate increases in current, and power density in supplemented cells ranged from 6 to 15 mW/m^2, as compared to 4 to 11 mW/m^2 in control cells. Calculations of electrons transferred in supplemented cells and chemical data suggest that sulfate reduction followed by sulfide oxidation at the anode remained the dominant anode process. Continued investigation of the cells' behavior will hopefully lead to a better understanding of factors that govern electron transfer reactions and current efficiencies.

Introduction

Microbial fuel cells have been considered a possible method of using biofuel for several decades.[1] In 2001, Reimers et. al introduced the first benthic microbial fuel cell (BMFC) that operated by spanning the natural redox gradient between marine sediment and overlying seawater.[2] Endogenous bacteria can oxidize organic matter by reducing oxidants in the sediment such as oxygen, nitrate and sulfate. By transferring electrons from an organic substrate to an electron acceptor at higher potential, bacteria gain energy.[3] If bacteria are in an anoxic environment where an anode is the electron acceptor at elevated potential, electron transfer will be primarily to the anode. If this anode is connected through an external load to a cathode in overlying seawater, the electrons obtained from reductants in the sediment can be moved through a circuit, generating current.

A clear application of microbial fuel cells is as a source of alternative energy. Thus far, the highest peak power density obtained from a BMFC is 1100 mW/m^2.[15] Power plants typically

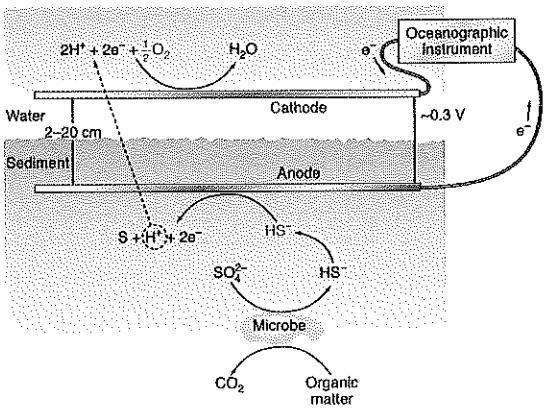
have an output on the order of a megawatt (10^9 mW), so powering a city with microbial fuel cells is not feasible yet. In Australia, researchers are attempting to scale up wastewater microbial fuel cells to produce kilowatts of power.[4] It is also possible, in the meantime, for BMFCs to power instruments, particularly environmental sensors used in oceanography. There is growing interest in long-term monitoring in remote environments and BMFCs could serve as a power source, negating the necessity of cruises to service installed instruments. With the monetary cost of a cruise reaching several hundred dollars per hour, an instrument with an indefinite power supply would be of great use to researchers.

While the BMFC has potential for application, power output is quite low, and several parameters can be investigated to optimize power production. Many sources of internal resistance decrease the power-generating potential of the cell. Electrical potential is lost through ohmic losses, activation losses, metabolic losses, and concentration losses.[5] The focus of this research is to understand anode processes in order to decrease anodic activation losses - those that occur in transferring electrons from the reductants to the electrodes.

The chemistry involved in BMFCs is a series of redox reactions catalyzed by microbes in the sediment and on the electrodes. The reaction begins at or near the anode, where species in the sediment are oxidized. There are two known methods of transferring electrons from bacteria to the anode. The first method is via soluble shuttles.[3] In this case, the bacteria reduce inorganic electron acceptors, producing reduced compounds, which are transferred to the anode through mediators produced by themselves or other bacterial species.[6] This is the method used by the sulfate-reducing bacteria (SRB) that become dominant at the anode in saltwater BMFCs.[7] These bacteria procure electrons from organic carbon through respiration, and are able to use those electrons to reduce sulfate, producing sulfide and water. The sulfide is then oxidized at the

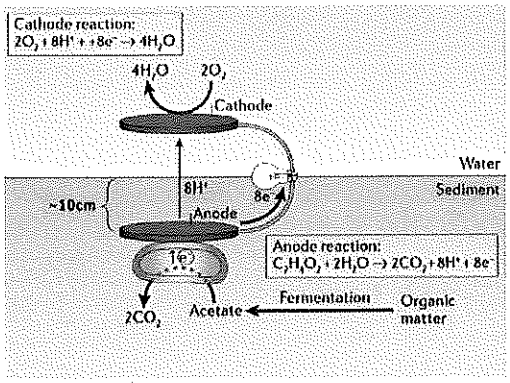
anode, moving electrons into the circuit. These electrons are transferred to oxygen at the cathode, completing the redox process.

Figure 1. *Redox reactions in seawater BMFC*[16]



In the second mechanism, electrons are transferred directly from the bacteria to the electrode, with no intermediate electron acceptors. The species *Geobacter* dominates in freshwater fuel cells[7] and is an example of bacteria that have membrane electron shuttling capabilities. This species can also oxidize fermentation products such as acetate and butyrate, which are common metabolic byproducts in sediments.[14] Since electrons are transferred directly to the anode, the theoretical coulombic efficiency of the redox process is higher than that for the SRB. We hypothesized that more efficient transfer of electrons would lead to a greater current density and increased power production (P=VI).

Figure 2. *Direct oxidation of carbon substrate in a BMFC*[17]



It is possible that an anode environment supplemented with organic matter will encourage the growth of extra-cellular electron shuttling bacteria (EESB) such as *Geobacter sulfurreducens*. If a carbon substrate is added to an SRB-dominated anode chamber where sulfate is limiting, EESB could take over. Recently, Rezaei et al supplemented small-scale BMFCs with the particulate substrates chitin and cellulose.[8] Power densities of about 70 mW/m^2 were achieved with the chitin, and 98 mW/m^2 for the cellulose. The coulombic efficiency of these cells was low (13% and 10%, respectively), a phenomenon attributed to the possibility that not all substrate had been used. Other supplementation work in sediment fuel cells has been done with an organic acid such as acetate as the substrate.[9] Lactate was chosen for this experiment because many organisms are known to grow on it as a carbon source and it could eventually be diffused in the field with Hydrogen Release Compound® (Regensis, San Clemente, CA). Since the primary mechanism for mass transfer in BMFCs is diffusion, a soluble substrate allows for a more kinetically favorable reaction than a particulate substrate.

The most favorable reactions in the fuel cell due to lactate addition could be:

a) $C_3H_6O_3 + 3H_2O \leftrightarrow 3CO_2 + 12\ e^- + 12\ H^+$

b) $3\ O_2 + 12\ H^+ + 12\ e^- \rightarrow 6\ H_2O$

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c) $C_3H_6O_3 + 3\ O_2 \rightarrow 3\ CO_2 + 3\ H_2O$

The oxidation reaction at the anode is shown through equation a), equation b) is the reduction reaction at the cathode, and c) is the net redox reaction. As shown by these reactions, twelve moles of electrons can be delivered to the circuit for each mole of lactate added.

The reactions that could result from sulfate reduction are:

a) $2\ C_3H_6O_3 + 3\ SO_4^{2-} + 3\ H^+ \rightarrow 3\ HS^- + 6\ CO_2 + 6\ H_2O$

b) $3\ HS^- \rightarrow 3\ S^\circ + 3\ H^+ + 6\ e^-$

Equation a) is mediated by microbes, and equation b) represents a chemical oxidation of sulfide at the anode, generating three electrons for every mole of lactate.

By comparing the increase in current of supplemented versus control cells, the moles of electrons passed should indicate which process is supplying electrons to the anode.

The design for this experiment was a nested chamber fuel cell, where the anode was immersed in an anoxic chamber above the sediment. This allowed the anode to be constructed from a high surface-area carbon brush, the current optimal electrode material.[10] The sediment was sandy, as opposed to the anaerobic muds typically used for BMFCs. This sediment mimics expected conditions at planned field experiment sites.

Methods

Fuel cells

Electrodes were constructed from high surface-area carbon fiber brushes. Ten cm anodes and 20 cm cathodes were used. The titanium wire of the electrodes was soldered to copper wire and waterproofed using layers of waterproof electrical tape and Scotchkote (3M, St. Paul, MN). The resistance across each electrode was less than 1 Ω. An Ag/AgCl (3 M KCl) micro-reference electrode (Microelectrodes, Inc., Bedford, NH) was placed in the overlying water. Before installation, reference electrodes from each of the fuel cells were tested and found to be stable and measured potentials within 10 mV of each other. The whole-cell voltage, anode voltage and current of each fuel cell were monitored using a multi-channel data logger (Agilent Technologies, Santa Clara, CA). The whole-cell voltage was controlled by a passive potentiostat (North-West Metasystems, Bainbridge Island, WA), which was set to keep the potential between the anode and the cathode at 0.4 V. Measurements were made every ten minutes.

Sediment for the fuel cells was collected from Yaquina Bay, Oregon at low tide. Sandy sediment was coarsely sieved and homogenized, and then 3.5 L of sediment was added to each cell. The sediment was left to settle overnight in the cold room before we began circulating the overlying water. Fuel cells were kept in the 10° C cold room for the course of the experiment, and seawater was circulated through the six cells using a pump and valve system. The circulating overlying water was replaced with fresh seawater every two weeks.

Anode chambers were crafted from acrylic tubes with UHMW plastic lids. The anode chamber was 15 cm in height, and 9.5 cm in diameter. They were pushed into the sediment until a ~ 70 mL volume was left between the sediment and the lid. Chambers had a rubber septum in the lid for sampling and supplementing, and a Teflon-tape reinforced compression fitting for the anode cable.

Figure 3. *Anode chamber*
Anode chamber with O-ring sealed lid, carbon fiber electrode, septum for sampling and anode cable exiting chamber through compression fitting.

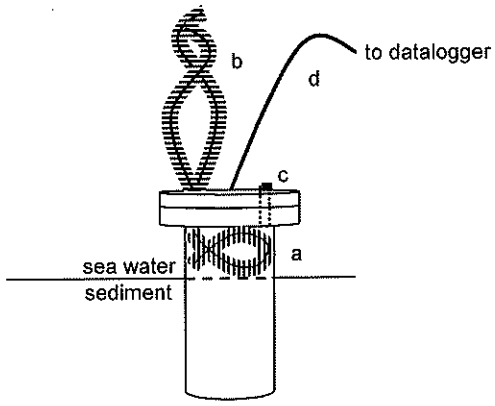


Figure 4. *Schematic of fuel cell*
Fuel cells were equipped with a) anode b) cathode c) septa and d) cable to data logger. See Appendix A1 for photograph of constructed cell.

Resistivity profiles of the sediment were measured with a Wenner array before the anode chamber was added to the cells. Profiles were measured in all six fuel cells, and then three extra profiles were recorded for cells 5 and 6. Measurements of resistivity were made every millimeter for 2.5 to 4 centimeters. These profiles were used to characterize the sediment and test for homogeneity of the six cells. In the future, these data will serve as a basis for comparison with resistivity profiles taken after the experiment.

Supplementation

Cells 2, 4, and 6 were supplemented with 1.0 mL 59.5 mM sodium lactate solution for a target concentration of 0.5 mM sodium lactate in each .07 L anode chamber. The solution was injected into the anode chamber through the septa with a plastic 5 mL syringe and 18-gauge hypodermic needle twice before circuits were closed, and then on day 7, 14, and 21.

Sampling

Sampling of each fuel cell's anode chamber as well as the cathode water was performed about once weekly. Samples were drawn through the septa using 10 mL gastight syringes and 18 gauge hypodermic needles. About 2 mL were sampled, then about 1 mL was expelled to release air bubbles. Anode water was then drawn to a volume of 11 mL, and then the syringe and needle were transferred to a glove bag. A 0.45 micron syringe filter (Paul, Ann Arbor, MI) was attached to the syringe, and 1 mL was expelled through the filter to rinse it. Seven mL were collected in a 15 mL Fischer vial, which was then partitioned in the glove bag in the following manner: 1 mL added to 20 μL zinc acetate for sulfide analysis, 2 mL for alkalinity and pH measurements, 0.500 mL for sulfate analysis, and remaining sample was frozen for future characterization by HPLC. The remaining 3.5 mL were filtered through a combusted glass microfiber filter (GF/F) into combusted glassware outside the glove bag and preserved for DOC analysis.

Analysis

To measure the sulfate in each sample, a turbidity method was used.[11] Samples were preserved until analysis in 1.5 mL microcentrifuge tubes at 4°C. Forty μL samples were mixed with 10 mL water, 0.5 mL 1 N HCl and 0.5 mL of a barium chloride/gelatin stock solution, then left to stand for thirty minutes. Turbidity was then measured with a 1 cm spectrophotometer zeroed to milli-q water. A standard curve was established using varying concentrations of IAPSO standard seawater (S= 34.996). Measurements were made in triplicate.

A Shimadzu TOC instrument was used to measure the concentration of dissolved organic carbon in the samples. Samples were preserved by adding H_3PO_4 to the ~3.0 mL of samples to a pH of 2. These samples were kept in the dark until analysis, at which point they were diluted to 10 mL and analyzed according to the non-purgeable organic carbon (NPOC) method. The mean area for the three injections was recorded and translated to concentration using a standard curve obtained from potassium hydrogen phthalate standards.

A pH electrode (Denver Instrument, Denver, CO) was used to measure the pH and alkalinity of anode chamber samples. A Gran titration was performed on the samples using 0.027 N HCl in 0.7 M NaCl.[12] Measurements of potential were taken and converted to pH using the electrode slope from a calibration with three buffers, and translated to alkalinity with the Gran function. Samples were not preserved, and all alkalinity measurements were made the day of sampling.

Sulfide concentration was determined spectrophotometrically.[13] A standard curve based on dilutions of a stock solution was established before each analysis. Standards were created in a glove bag and samples were treated with zinc acetate upon collection to prevent oxygenation. These treated samples were stored in the dark until analysis. Cline C (40-250 μM) was chosen as

the reagent in analysis of the anode waters for the first three weeks of samples, and Cline B (3-40 μM) was used for subsequent sulfide analyses.

Results

Power Generation

Supplementation of the fuel cells resulted in sharp increases in both current and power density (see Appendix). Power density (ρ_p, W/m^2) was calculated as $\rho_P = \frac{I \times V}{A}$, where I = current, V = whole cell voltage and A is the area of the footprint of the anode chamber (5.67 x 10^{-3} m^2). The range of power densities for a supplemented cell was 5.9 mW/m^2 to 15.3 mW/m^2, as compared to 3.7 mW/m^2 to 11.3 mW/m^2 for the control cells.

Current

Figure 5. *Current in six fuel cells.*

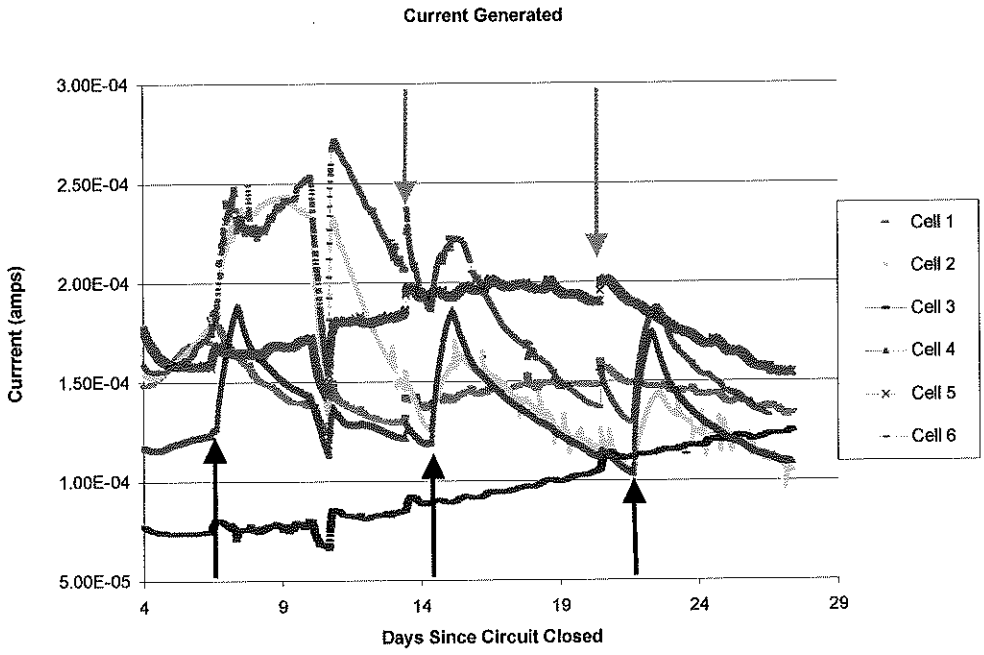


Figure 5 shows the currents for all six fuel cells for the course of the experiment. Supplementation was followed by a sharp increase in current for cells 2, 4, and 6 (black arrows). On day 10, the cathode water circulation system broke down and water drained from the cells.

This resulted in the sharp drop in current for all curves. Shortly afterwards, the water was replaced, restoring current levels. The increases in current on day 13 and day 20 are due to sampling (green arrows). The curve returns to its original path shortly after sampling.

The control cells (1, 3 and 5) had more stable current values over the twenty days. As of the end of data collection for this report, all three appeared to be a similar current level (~13 mA).

In the case of the supplemented cells (2, 4 , and 6), cell 4 had the most consistent response to the first three supplements. Based on the DOC data from the first supplement, cell 4 consumed the lactate more quickly than cell 2 and 6. This suggests that the microbial community in cell 4 was carbon limited before the first supplement. At the time when circuits were closed, cell 4 had the lowest open circuit potential of the three supplemented cells. Cell 2 and 6 had not yet reached limiting conditions in the anode, and their behavior in the first two supplements reflects the availability of reductants besides lactate. With subsequent supplementations, cells 2 and 6 responded to lactate addition in a fashion more similar to cell 4. In cells 2 and 6, therefore, background processes skewed the effect of the first two supplementations. By the fourth supplement, we expect organic carbon to be limiting and predict the behavior of cell 2 and 6 to follow that of cell 4.

Electrons passed

We focused on cell 4 when analyzing current to determine which of the two electron transfer processes was occurring at the anode. This is the cell that exhibited the most consistent behavior in the first few weeks of this experiment, and the following calculations will be applied to cells 2 and 6 in future weeks as they stabilize.

Moles of electrons passed through the circuit can be calculated from the current measured by the data logger as $M = \frac{I\Delta t}{F}$ where I = current, Δt = time interval, and F = Faraday's constant (96,485 coulombs/mole). This is effectively taking the integral of the current curve. It was approximated that current would have varied linearly in the absence of supplementation, and a line was constructed from the point directly before supplementation and the point before the next sampling. The difference between the current curve and this line was used to determine how many moles of electrons passed due to lactate supplementation.

Figure 6. *Current in Cell 4 after Second Supplementation*

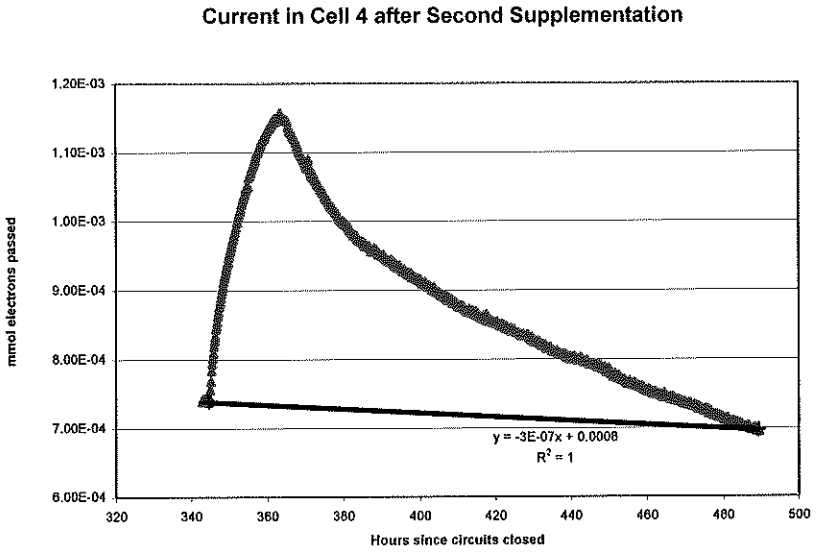
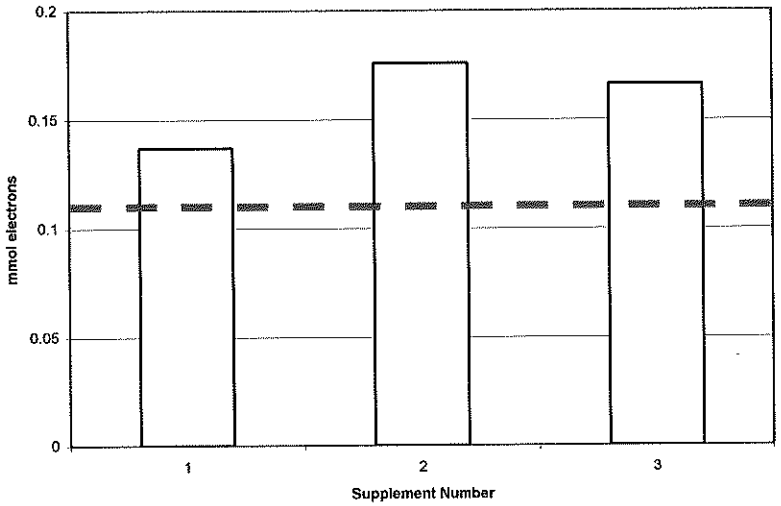


Figure 6 shows the current in cell 4 after supplementation. The moles of electrons passed due to lactate addition were found by determining the area of the curve between the current curve and the line which projects current in the absence of supplementation.

As 0.035 mmol lactate was added during each supplementation, the theoretical moles of electrons passed from direct oxidation of lactate was 0.035 mmol lactate *(12 mol electron/mol lactate) = 0.42 mmol. For the indirect sulfate reduction pathway, 0.11 mmol electrons were expected.

Figure 7. *Electrons passed by cell 4*



In Figure 7, the dotted line represents the expected number of electrons if transfer is through sulfate reduction.

All supplements exceeded the theoretical number of electrons passed if sulfide reduction to sulfur, following sulfate reduction, was the only process occurring at the anode. Yet the inventory of electrons passed was much closer to 0.11 mmol than 0.42 mmol, suggesting that sulfate oxidation continued to be the main process even after supplementation. The excess electrons are likely due to consumption of carbon naturally present in the sediment; initial DOC concentrations were up to twice as high as expected due to lactate supplementation. Though we attempted to eliminate background processes by subtracting the projected current line, comparison to control cells and a better approximation of background would give a more accurate value of electrons transferred solely from the lactate added.

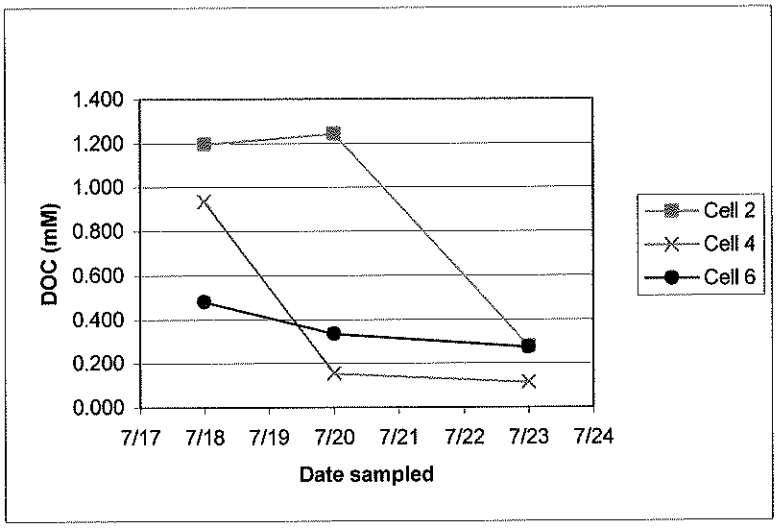
Chemical parameters

DOC

As expected, the concentration of DOC in the supplemented anodes was initially higher than that of the control anodes, with the exception of cell 6 which had a lower concentration than

control cell 1 (see Figure 9). Since anode chamber volume is quite approximate (and did not account for the volume of the anode itself) it is possible that cell 6 had a larger anode chamber volume than expected and thus had a lower concentration. Initial values are greater than the target 0.5 mM concentration mostly likely due to biomass in the sediment. The concentration of DOC in the cells decreased between the first and second samplings, and by seven days after supplementation, DOC concentrations in the supplemented cells were effectively the same as those in the controls, suggesting that all lactate had been consumed at this time. Cell 4 notably consumed lactate more quickly than cell 2 and 6, giving evidence of a stable microbial and chemical environment. The control cells had more variable concentrations of DOC throughout the sampling period, possibly due to POC hydrolysis. Further analysis of DOC for the second and third supplementations will shed light on whether or not cells 2 and 6 are also stabilizing.

Figure 8. *DOC in supplemented fuel cells.*



Sulfate

Control cells 1, 3 and 5 exhibited similar sulfate concentrations between days 1 and 20. An increase in sulfate concentration occurred shortly after circuits were closed, which could have

resulted from pore water entering the anode chamber because of sampling. Sulfate was then reduced to sulfide, decreasing its concentration in the anode chambers of all six cells (see A2).

Sulfide

Concentration of sulfide was higher in all three supplemented cells than their control counterparts (see A3). This suggests that SRB were more active in these cells than the control, as more carbon substrate was available for respiration. Towards the end of the sampling period, supplemented cells exhibited an increasing concentration of sulfide while the control cells decreased, which could be the result of slower uptake of HS^- at the anode (supporting the direct oxidation mechanism) or accelerated production of HS^- through sulfate reduction.

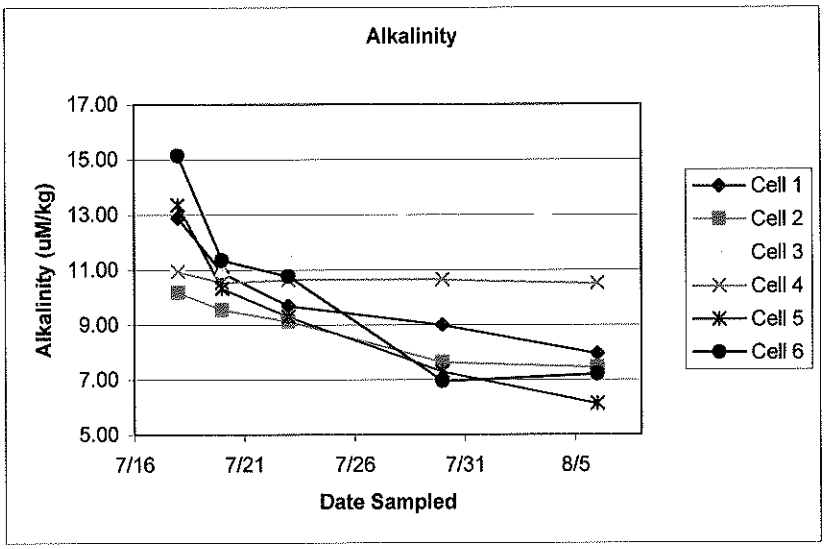
pH

Overall, the pH of all fuel cells decreased (see A4). Hydrogen ions are produced at the anode in both the shuttling SRB mechanism as well as the membrane EESB one.

Alkalinity

Alkalinity also decreased. This follows from the production of H^+ ions by anode reactions. Alkalinity would be increased by sulfate reduction to sulfide, and could also be buffered by the dissolution of calcium carbonate from sediment. Most notably, the alkalinity of cell 4 was constant over the experiment. This is the behavior expected for a cell that is primarily undergoing sulfate reduction, because sulfide ions created from sulfate reduction are taken up by the hydrogen-ion producing reaction at the anode. The alkalinity of the water would stay relatively balanced. In the case of direct lactate oxidation, no alkaline ions are produced. The formation of excess H^+ ions is not balanced by another reaction and alkalinity would decrease.

Figure 9. *Alkalinity in fuel cells*



Miscellaneous

The sampling volume was about 12 mL, which accounts for a sixth of the total volume of the anode chamber. It was assumed that this volume of water was replaced by pore water, which could have changed the concentration of species in the anode chamber. While the data suggests that the electricity trends continued as they would have without sampling, the chemical analysis information (particularly in the case where concentrations decreased) may be skewed by the removal of anode water. A larger fuel cell that would be less affected by sampling could give more reliable information on chemical composition of the anode waters.

The time-scale of this experiment including setup was ten weeks, and there was not enough time to let the fuel cells stabilize before supplementation and measurement began. Not all six cells had fully reached open circuit potential at the time that circuits were closed. The data from the first three supplements suggests that the cells were continuing to stabilize, and may show clearer patterns in coming weeks. Further supplementation and sampling will clarify the nature of the anode processes. Other interesting observations to consider are whether or not the effect of supplementation strengthens or weakens, how the behavior of the sandy sediment fuel

cell compares to anoxic mud cells, and what changes occur in the resistivity profiles of the cells. An analysis of the microbial communities present in the control and supplemented cells could confirm whether or not the supplementation encouraged growth of EESB over SRB.

Conclusions

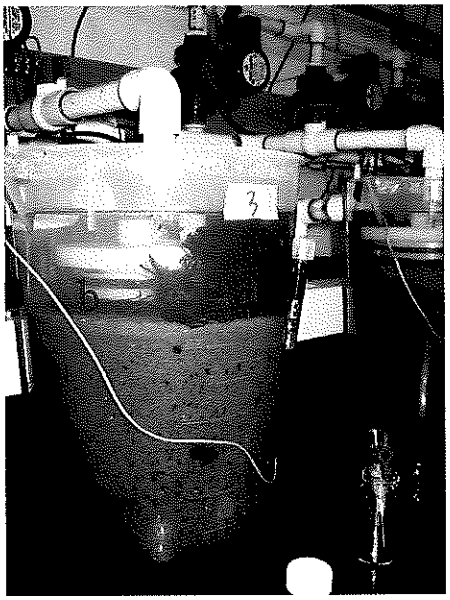
Supplementation increased current and power density in all three fuel cells. Chemical and electrical data from supplemented cell 4 tend to show that sulfate reduction followed by electrochemical sulfide oxidation is the main mechanism of electron transfer to the anode, but further collection of data, in particular from the other supplemented cells, is necessary to justify this conclusion. Much of the information collected in the first few weeks could have been influenced by settling processes in the fuel cells. If supplementation with a soluble substrate such as lactate is to be used for BMFCs deployed in the field, a method to slowly release the lactate must be researched. We are currently testing the use of Hydrogen Release Compound ® and a diffusion membrane in seawater. As improvements to the design, biological and material aspects of BMFCs accumulate, supplementation may play a role in increasing the lifetime of fuel cells and their consequent relevancy as a viable source of energy.

Acknowledgements

Funding for this experiment was provided by a NSF Research Experience for Undergraduates Internship under award OCE-0648515 to the Hatfield Marine Science Center of Oregon State University, and the Office of Naval Research. I would like to thank Clare Reimers and Mark Nielsen for their guidance and support throughout the summer, and Jessica Martinez for help with sampling.

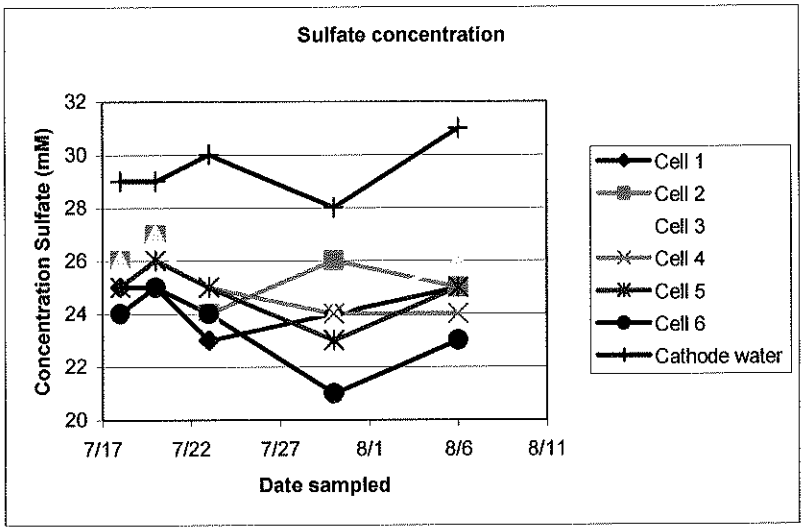
Appendix

A1: Photograph of fuel cell

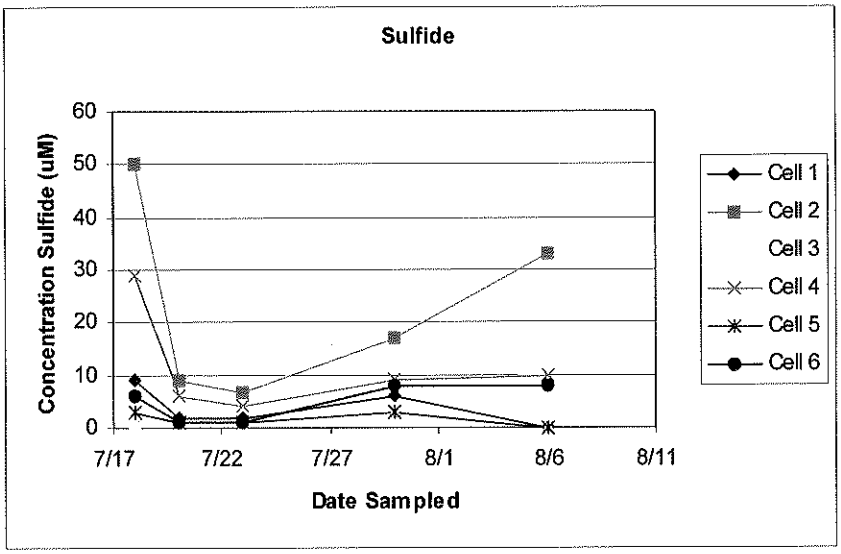


One of six fuel cells constructed for the experiment. The cathode is in the circulating overlying water, labeled **a**, and the anode chamber is in the sediment, labeled **b.**

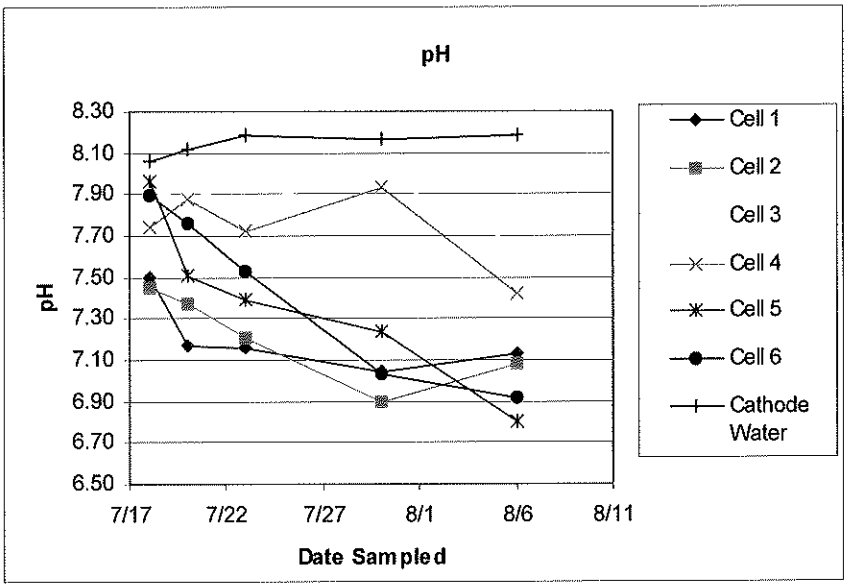
A2: Sulfate concentration



A3: Sulfide concentration.



A4: pH



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